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Cell-specific expression profiling of rare cell-types as exemplified by its impact on our understanding of female gametophyte development

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Expression profiling of single cells can yield insights into cell specification, cellular differentiation processes, and cell type-specific responses to environmental stimuli. Recent work has established excellent tools to perform genome-wide expression studies of individual cell types, even if the cells of interest occur at low frequency within an organ. We review the advances and impact of gene expression studies of rare cell types, as exemplified by recently gained insights into the development and function of the angiosperm female gametophyte. The detailed transcriptional characterization of different stages during female gametophyte development has significantly helped to improve our understanding of cellular specification or cell-cell communication processes. The next-generation sequencing approach – used increasingly for expression profiling – will now allow for comparative approaches that focus on agriculturally, ecologically or evolutionarily relevant aspects of plant reproduction.

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Highlights:

- Recent technological advances enable single-cell transcriptomics of rare cell types

- Research on female gametogenesis has strongly profited from such technologies

- Reproductive cells have been characterized by transcriptomics in model species

- RNA-Seq will allow comparative evolutionary approaches to reproductive processes

Introduction

Cell specification, cellular differentiation, and specific cellular responses to environmental stimuli involve changes in gene expression. Therefore, a view of the transcriptome of a cell provides a snapshot of the cellular instruction machinery that strongly depends on developmental stage and environmental inputs. Recent technological developments have enabled genome-wide expression experiments at reasonable costs [1]. In addition, cell type-specific transcriptional profiling has dramatically improved our understanding of biological processes [e.g. reviewed in 2]. Two major lessons have been learned from the analysis of genome-wide expression data in individual cell type or specific tissues in plants:

First, the cellular context is important when studying developmental processes, because cell-specific gene expression is generally masked when performing studies at the organ level (reviewed in [3]). Ground-breaking novel insights into the development of plants have been made by profiling essentially all cell types that occur in the *Arabidopsis* root [4-7], through studying male gametophyte development (pollen) [8], or by expression profiling of *Arabidopsis* female gametophyte (embryo sac) development [9, 10*, 11**, 12**] (see also below).

Second, multiple lines of evidence suggest that not only cellular differentiation processes are best understood at the single-cell level, but also responses of an organism to environmental stimuli: in genome-wide expression studies strong interactions between cellular identity and environmental variation have been observed, for example when examining the effects of stress or nutrient treatments on different cell types in the root [13-15].

Here, we summarize the tools that are available to isolate specific cell types from heterogeneous tissues as well as advances in transcriptional profiling methods. Additional reviews on related topics have been published recently [2, 3, 16, 17], but here we briefly summarize and compare the tools with a focus on their suitability for cells that occur at low frequencies within a tissue. We also discuss what insights have been gained through cell-specific gene expression profiling of rare cell types in plants, as exemplified by studies on female reproductive processes.

Techniques used for the isolation of individual cell types

Initial approaches for genome-wide expression profiling largely focused on the profiling technologies themselves, meaning that experiments were often performed at the whole-plant, organ, or tissue level [18]. Only for certain cell types, such as those of the male gametophyte, could specific stages be collected and profiled relatively easily [8]. In recent years however, the scientific community has been creative in generating a variety of methods to isolate or profile distinct cell types at specific developmental stages. Here, we summarize recently applied approaches – and identify limits, strengths and weaknesses of these – with a special focus on their application to the collection of rare cell types (**Table 1**). For the use of methods in applications using frequent cell types, we refer to previous recent reviews [2, 3, 19].

a) Biochemical purification of selected cell types. It is possible to isolate certain cell types using mechanical and/or biochemical enrichment procedures. This approach is only applicable to selected cell types, e.g. guard cells, trichomes and sperm cells, with specifically designed methods for each [20-22]. Since it is possible to isolate relatively large numbers of cells using such procedures, they are not only suitable for transcriptomics [21, 22], but also for proteomics [20, 23-25] metabolomics [26], analyses of DNA methylation [20, 27], or the determination of cell wall composition [22].

b) Genetic subtraction methods. Hereby, a genetic background that alters developmental processes or cell type abundance in a tissue is employed, for instance mutants with an increased number of stomata [28], mutants with altered floral organ [29], floral mutants in combination with inducible transgenic constructs [30, 31], or mutants missing the female gametophyte [32-35]. These tools are fairly limited to the biological system under study and often rely on specific mutants and/or transgenic backgrounds that may be difficult and time-consuming to establish. Furthermore, the approach is subtractive and, by definition, genes that are expressed both in the surrounding tissue and the target cells cannot be detected.

c) Fluorescence-activated sorting of cells or nuclei. The approach relies on automatically sorting cells

[4, 7, 36] or nuclei [37] that are tagged with a fluorescent marker (such as the Green Fluorescent Protein) using a flow cytometry system. The method results in high yields and good enrichments, and is suitable for transcriptomics [4, 7, 36, 38], proteomics [39], and metabolomics [3]. However, the method is usually limited to cells that have a relative occurrence of more than 0.5% (0.1% under highly optimized conditions) within the harvested tissue (*Kenneth Birnbaum, personal communication)*. This requirement impedes, e.g. the isolation of female gametophytic cells from carpels or even from isolated ovules.

d) Cell-specific tagging of RNA, RNA-binding proteins, or components of the ribosome. In several experiments, specific tagging and pull-down of either RNA (e.g. in *Drosophila* [40]), RNA-binding proteins, or ribosomal components [41-44] has yielded insights into cell-specific processes. For example, in a study of cell-specific responses to environmental variation, it was shown how hypoxic stress affects the translatome (i.e. the mRNA population associated with the ribosome in the process of translation) [44]. However, these methods have so far been used for more frequently occurring cell types only, and their use for rare cell types may not feasible [40].

e) Affinity-isolation of nuclei from specific cell types. A recently established, elegant method for cell-specific expression profiling is INTACT, the Isolation of Nuclei TAgged in specific Cell Types [45**, 46]. The method relies on affinity purification of nuclei that carry a biotinylated fusion protein in their nuclear envelopes. Thus, it supersedes the need to isolate whole cells, and offers a cheap and simple alternative to performing expression profiling on specific cell types. It has been used in model organisms such as *Arabidopsis* or *C. elegans* [47]. A further development of the method yielded excellent results for isolating subpopulations of *Drosophila* brain cells [48**]. In the latter work, enrichments of around 95% were achieved from tagged nuclei in neuronal cells that only make up around 0.14% of the *Drosophila* brain. Such a technique would make it possible to work with even some of the rarest cell types in *Arabidopsis*, and also allow the study of chromatin features in these cells. The technology is still being optimized and future work will reveal it is useful for the study of megagametogenesis. Like fluorescence activated cell sorting the INTACT system relies on genetic transformation and the use of suitable promoters that are active only in certain cell-types.

f) Microsurgical manipulation. This method relies on the use of micropipettes to isolate single cells dissociated from heterogeneous tissues. It has been applied to the isolation of various rare cell types, such as female gametophytic cells from diverse plant species (see also below) [49-51]. The method has a relatively low yield but can be used to isolate rare cell types as long as they can be distinguished from the surrounding cells. Although it is easier to recognize the cells as they are marked, this method does not absolutely require the use of transgenic lines and is therefore applicable to cell types for which no specific promoters are available, as well as to non-model organisms that cannot be easily transformed.

g) Laser-assisted microdissection (LAM). This approach involves the isolation and extraction of specific tissue compartments [52-59], or even individual cell types [9, 10*, 11**], with the use of a laser. Hereby, a tissue containing the cells of interest is chemically fixed, embedded in resin, and sectioned on a microtome. Cells are then isolated based on morphology and cytology. This allows the isolation of rare cell types within complex tissues and even the isolation of subcellular domains, e.g. specific regions within syncytial structures such as the endosperm [56] or embryo sac (Schmid and Grossniklaus, unpublished). However, yields are often limited and the use of LAM is usually restricted to transcriptomics, although it has also been used for the analyses of cell type-specific DNA methylation patterns [27, 60]. This method does not require the generation of transgenic lines but the cells to be isolated have to be distinguishable in a section.

Cell-specific expression profiling – insights into female gametophyte development

The gametophytic generation in higher plants is of great agricultural importance. For instance, male-sterile plant lines can be generated for use in hybrid seed production through genetic or transgenic manipulations [61] or, in the future, apomictic crop species could be developed that would revolutionize breeding efforts [62, 63]. However, a better understanding of the molecular basis of gametophyte development, which would open avenues for its manipulation for uses in crop breeding, has been hampered by its small size and, in particular for the female gametophyte, its inaccessibility. In the course of evolution, the gametophytic generation has become more and more restricted, such that in angiosperms it is represented as highly reduced organism, consisting of a few cells only, which is dependent on the sporophyte. Typically, male and female angiosperm gametophytes consist of only

three and seven cells at maturity, respectively (**Figure 1**) [64].

Due to its simple organization and polar structure, the female gametophyte is considered an excellent system to study fundamental developmental processes, such as pattern formation, cell specification, and cell-cell communication [65-67]. However, the embryo sac of angiosperms is a very small structure, deeply embedded in sporophytic tissues, making it inaccessible for expression profiling studies. Classical genetic approaches have advanced our view of the molecular bases of developmental processes during gametogenesis [66, 68]. Despite its success, the genetic approach has its shortcomings and only a few of the identified genes have been characterized in detail. To get a comprehensive overview over the genes involved in female gametophyte development it is thus desirable to use the advantages of current gene expression profiling tools to study embryo sac development.

The earliest gene expression studies used female gametophytic cells obtained by micromanipulation and subsequent EST-sequencing, first in maize [49] and later also in tobacco [69], wheat [70], and *Torrenia fournieri* [51]. Important insights into the communication between male and female gametophytes [50, 51] and cellular specification [71] were only made possible through these EST-sequencing projects. For example, a recent EST-sequencing study in wheat revealed that RWP-RK domain-containing transcription factors exhibit egg cell-specific expression [72*]. The study also showed that two *Arabidopsis* homologues, *AtRKD1* and *AtRKD2*, could induce an egg cell-like transcriptional program when ectopically expressed in sporophytic tissue. Interestingly, the RKD transcription factors show similarity to the *MINUS DOMINANCE* gene of the green algae *Chlamydomonas reinhardtii*, which is important for sex-determination [73]. Even though loss-of-function phenotypes in *Arabidopsis* have not been described for *AtRKD1* or *AtRKD2* because of genetic redundancy, it is likely that their gene products are involved in the specification of the female gametes as evidenced by their ability to reprogram the transcriptional profile of sporophytic cells towards an egg cell fate [72*].

Extensions to these EST-sequencing projects include recent efforts that use a combination of single-cell isolation and microarray profiling [9, 11**, 12**, 74*] or the sequencing of RNA based on next generation sequencing-based methods (RNA-Seq – see also next paragraph) [10]. These approaches

have provided a more comprehensive view of the transcriptome and its dynamics during megagametogenesis. For example, profiles of an extensive selection of reproductive cell types in *Arabidopsis* as measured by the Affymetrix ATH1 GeneCHIP are available (**Figure 2a**). These data are an important resource to the research community, for example for reverse genetics [11**, 12**] or to support the map-based cloning of mutant loci [75, 76]. These studies also revealed that the transcriptomes of gametophytic cells contain a lot of uncharacterized genes and gene families (**Figure 2b**), some of which have most likely been missed during the development of the most popular microarray platforms [10*] (see also below). Thus, gametophytic cells express a large fraction of genes for which no function is known and their genetic characterization will reveal their roles during gametogenesis. Of course, such a study can be hampered by genetic redundancy, as was recently revealed for an egg cell-specific family of secreted peptides. Only the mutation or down-regulation of all five copies of this gene family revealed the specific function of these peptides in the fertilization process [77].

Such microgenomics approaches can be tied in with a functional framework on the mechanisms underlying development. A nice example of how transcriptional profiling can enhance our understanding of these - and resolve apparent discrepancies between different experimental observations - was recently published [12**]: it had been suggested that small RNA pathways are important for female gametophyte development and germline specification. The female germline is initiated with the determination of the MMC and terminates with the differentiation of the egg in the mature embryo sac [78]. In the first LAM-based gene expression map of the mature female gametophyte in *Arabidopsis* [9], it was found that genes of the *ARGONAUTE* (*AGO*) family, associated with small RNA pathways, are strongly expressed in the egg cell, suggesting an important role in the female gametophyte. On the other hand, mutations in the *AGO9* gene had been reported to result in in ectopic formation of megaspore mother cells (MMCs) [79**], suggesting that small RNA pathways are important in opposing female germline specification in somatic tissues. Thus, it was unclear whether small RNA pathways were involved in promoting or opposing megagametophyte development. Applying LAM to identify genes expressed in the nucellar region of the ovule that contains developing megaspores, *AGO5* was found to show elevated expression [12**]. Indeed, a semidominant mutant allele of *AGO5* - which is an effector of small RNA pathways - results in a failure to initiate

megagametogenesis. Similar defects were observed by expressing inhibitors of small RNA pathways in the nucellar region of the ovule. Therefore, there are apparently two opposing small RNA pathways that restrict MMC specification to a single cell in the ovule and promote the development of the female gametophyte, respectively [12**, 79**].

Next generation sequencing technologies – an unbiased and flexible toolkit to allow comparative studies in non-model organisms

In the past ten years, microarrays have proven indispensable tool for transcriptional profiling of rare cell types. The growing collection of publicly available data (e.g. based on the Affymetrix *Arabidopsis* ATH1 GeneCHIP), offers the opportunity to not only describe a newly obtained transcriptome, but also identify cell type-specific expression patterns – the essence of cell differentiation [80]. Nonetheless, microarrays are constrained by the underlying technology and their design: (i) hybridization-based measurements can exhibit high background levels due to cross-hybridization, and generally lack sensitivity at low and high expression levels; and (ii) the design relies upon existing knowledge of the genome sequence [1]. The latter is of particular importance for transcriptome arrays, which can become outdated regarding transcriptome coverage. For example, the ATH1 array lacks probes for ~36% of all genes, pseudo-genes, and transposable element genes annotated in TAIR10 [10*]. Intriguingly, a considerable fraction of these is likely to be important for reproductive development [10*, 32, 35].

An alternative that overcomes these limitations is RNA-Seq [1], which has been shown to be sensitive in detecting transcripts in rare cell types [10*, 81, 82]. In contrast to most microarray platforms, it also allows for a less biased analysis of the transcriptome and enables the identification of previously unannotated loci or transcript variants [83]. For example, a 2-3 times higher fraction of reads aligning uniquely to introns, regions flanking annotated loci, and isolated intergenic regions was observed in the central cell of *Arabidopsis* as compared to other RNA-Seq transcriptomes (16%, 7%, 7%, and 3.5% in central cells [10*], male meiocytes [82], pool of organs and seedlings [84], and unopened flower buds [85], respectively). This likely indicates novel transcribed regions and transcript variants that are specific to the central cell [10*].

RNA-Seq also offers the opportunity to study organisms that lack reference sequences. It may therefore promote the use of non-model species to study diverse ecologically, evolutionarily, and agriculturally relevant plant traits (reviewed in [86]). Depending on the organism and the availability of sequence information from closely related species, the analysis strategy may either be to (i) align the reads to the reference sequences of a related species and use the annotation directly [87]; (ii) use those alignments for a reference-guided assembly [83]; or (iii) perform a *de novo* assembly [88]. In many cases single cell specificity in non-model organisms may only be achieved with isolation methods that do not rely on the generation of transgenic plants, for example with microdissection or LAM (see example in [89]). For our understanding of the gametophytic generation in higher plants, and extension of expression profiling methods to non-model organisms bears great promises for breakthroughs. For instance, recent efforts of characterizing the molecular bases of apomixis, the asexual reproduction through seeds, are increasingly focused on expression studies for a comparison of asexual with sexual reproduction [62]. Another example is the comparative approach taken to reveal the transcriptomes of the gametophytic and sporophytic generations in the water moss *Funaria hygrometrica*. This comparison revealed a weaker differentiation in gene expression between the two generations compared to in *Arabidopsis*, which was attributed to the fact that both generations of *F. hygrometrica* are well developed and consist of a large number of cells, whereas *Arabidopsis* has dramatically reduced gametophytes [87].

Expression studies in individual cell types and under selected conditions have already revealed exciting insights into plant development and cell type-specific responses to environmental stimuli. A cell type-resolved view of gene expression in several plant species is now in reach, and will allow for comparative studies that will shed new light onto the evolution of developmental processes, including that of sexual and asexual reproduction.

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Figure Legends

Figure 1

Schematic representations of the mature male and female gametophyte. (a) The male gametophyte, the pollen, develops mitotically from the microspores formed in the male floral structure, the anther. It contains a vegetative cells and two sperm cells, the latter of which fertilize the two female gametes (central cell and egg) in a process termed double fertilization. (b) The female gametophyte, also termed embryo sac, is a seven-celled structure that develops within the sporophytic tissue of the ovule. It is made up by four different cell types, namely three antipodals (that can degenerate during the final stages of megagametogenesis), two synergids (accessory cells critical to the fertilization process), a homodiploid central cell which is fertilized by one sperm cell to give rise to the triploid endosperm and an egg cell which is fertilized by the second sperm to give rise to the zygote.

Figure 2

Gene-sharing network of reproductive cell types in *Arabidopsis* as determined by a collection of experiments using the Affymetrix ATH1 GeneCHIP. **(a)** Gene-sharing network [80] showing a selection of reproductive cell types transcriptomes. Edge weights denote the number of genes shared between two nodes. Node size is proportional to the number of genes that are specifically enriched in a given cell type. **(b)** Selected PFAM-domains that are overrepresented amongst gene products specifically expressed in different reproductive tissue (as determined in A). Color scale denotes p-values (with darker colors denoting smaller p-values), domain of unknown functions are highlighted in red.

Table 1: Summary of popular cell isolation methods developed in recent years.

Cell isolation method	Limits of relative cell occurrence	Relative enrichment scores of cells**	Technique/ Costs	Further applications	Use in non-model organisms?
Biochemical isolation	N/A [21, 22]	N/A	Cheap	Many	Yes, but limited to few, selected cell types
FACS	~1% (down to 0.1% if highly optimized)	~9-60x [45, 90]	Expensive equipment, extensive protocol	Proteomics, metabolomics, genome-wide chromatin structure	Relies on transgenic lines
LAM	<0.1%	Depends on morphology (up to ~ 10,000x) [9]	Expensive equipment, long protocol	For rare cells: Mostly limited to expression profiling (low throughput) but has also been used for DNA methylation analyses	Yes
INTACT/INTACT-derived	<1% - 10%*	100-170x [45**]/ up to 10,000x [48**]	Easy protocol, cheap	Genome-wide chromatin structure	Relies on transgenic lines
Micromanipulation	<0.1%	Depends on accessibility of cells (~up to 10,000x) [49]	Technically challenging, depends on morphology of cell type	For rare cells: Limited to expression profiling (due to low throughput)	Yes

* the limits of the method have not been tested thoroughly

** relative enrichment scores are defined here as [no. of target cells in output / no. of non-target cells in output] / [no. target cells in input / no. of non-target cells in input], e.g. from 10% relative fraction in input to 99% relative fraction in output: enrichment score = (99 / 1) / (10 / 90) = 891.

Figure 1

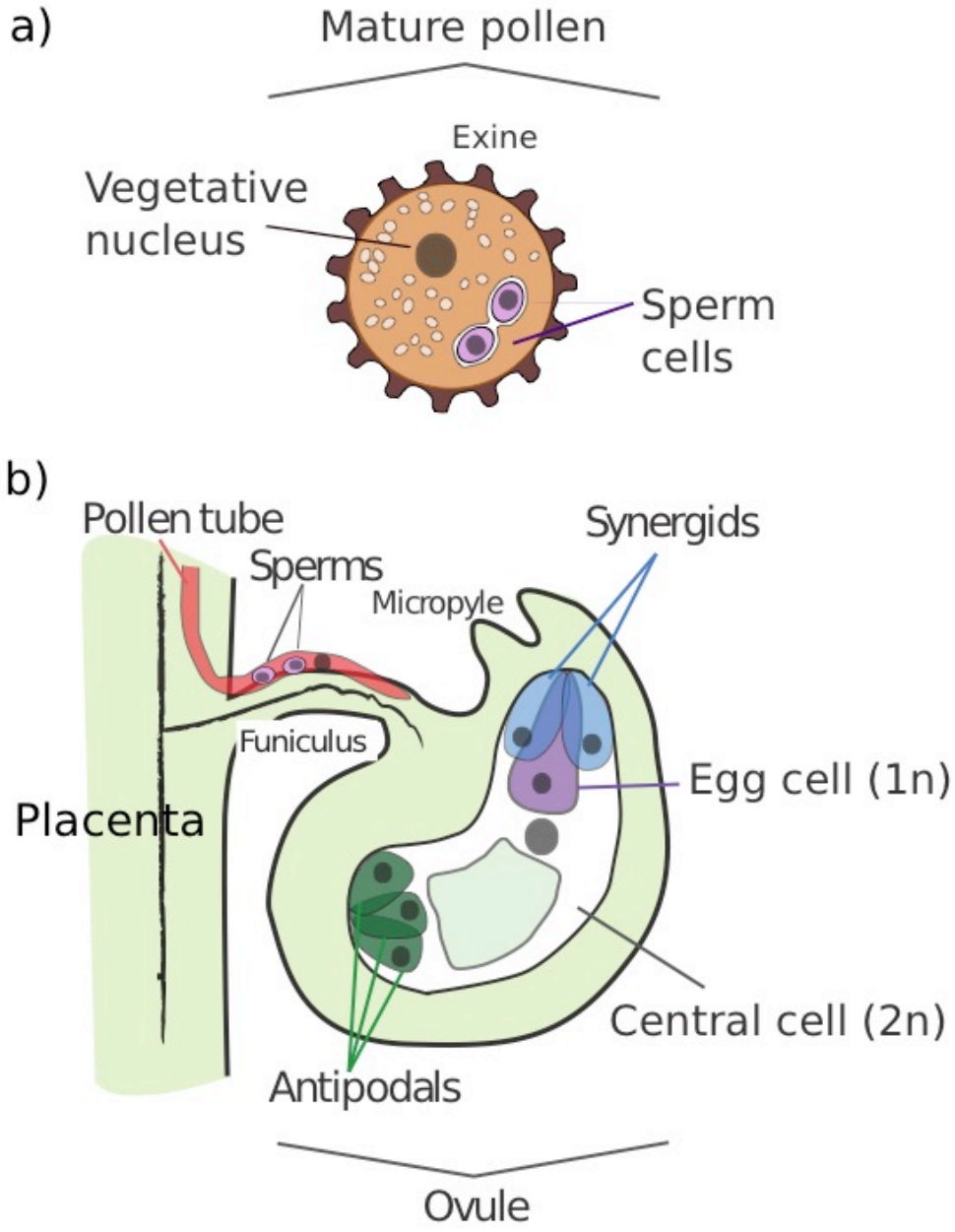
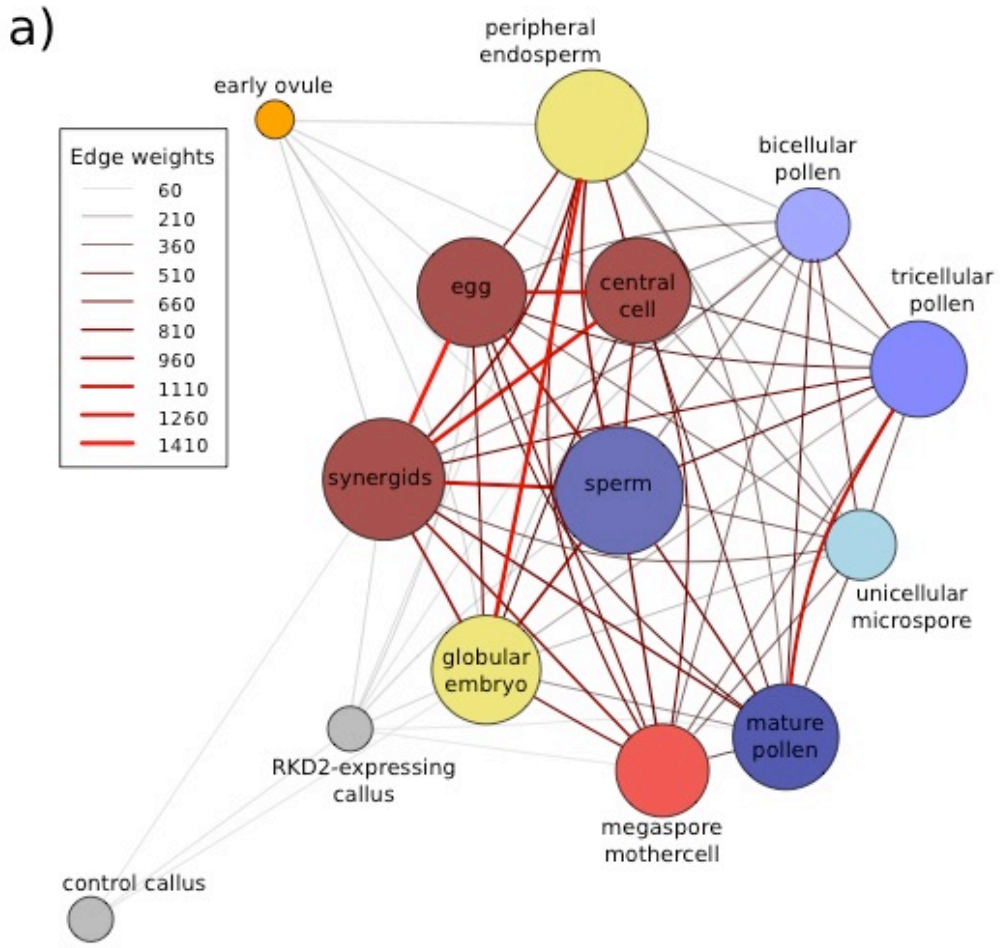
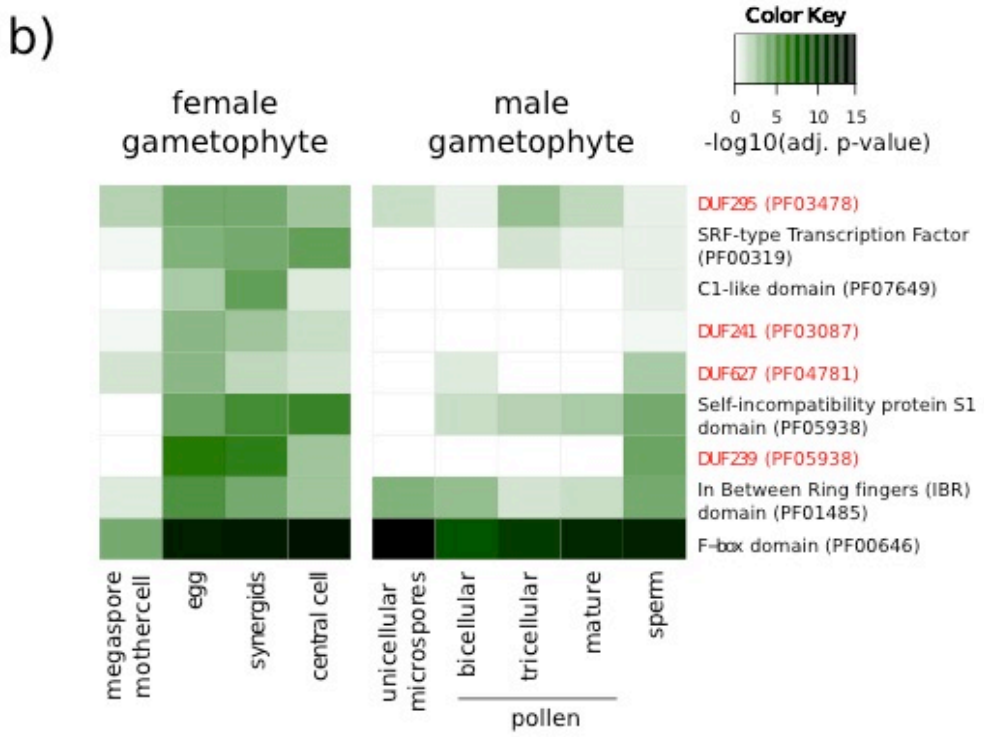

a)
Mature pollen
Exine
Vegetative nucleus
Sperm cells
b)
Pollen tube
Sperms
Micropyle
Synergids
Funiculus
Egg cell (1n)
Placenta
Central cell (2n)
Antipodals
Ovule

Figure 2


a)
peripheral endosperm
early ovule
Edge weights
60
210
360
510
660
810
960
1110
1260
1410
bicellular pollen
egg
central cell
tricellular pollen
synergids
sperm
unicellular microspore
globular embryo
mature pollen
RKD2-expressing callus
megaspore mothercell
control callus


b)
Color Key
0 5 10 15
-log10(adj. p-value)
female gametophyte
male gametophyte
DUF295 (PF03478)
SRF-type Transcription Factor (PF00319)
C1-like domain (PF07649)
DUF241 (PF03087)
DUF627 (PF04781)
Self-incompatibility protein S1 domain (PF05938)
DUF239 (PF05938)
In Between Ring fingers (IBR) domain (PF01485)
F-box domain (PF00646)
megaspore mothercell
egg
synergids
central cell
unicellular microspores
bicellular
tricellular
mature
sperm
pollen

References

[1] Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 2009; 10:57-63.
[2] Taylor-Teeples M, Ron M, Brady SM. Novel biological insights revealed from cell type-specific expression profiling. Curr Opin Plant Biol 2011; 14:601-7.
[3] Rogers ED, Jackson T, Moussaieff A et al. Cell type-specific transcriptional profiling: implications for metabolite profiling. Plant J 2012; 70:5-17.
[4] Brady SM, Orlando DA, Lee JY et al. A high-resolution root spatiotemporal map reveals dominant expression patterns. Science 2007; 318:801-6.
[5] Moreno-Risueno MA, Van Norman JM, Moreno A et al. Oscillating gene expression determines competence for periodic Arabidopsis root branching. Science 2010; 329:1306-11.
[6] Iyer-Pascuzzi AS, Jackson T, Cui H et al. Cell identity regulators link development and stress responses in the Arabidopsis root. Dev Cell 2011; 21:770-82.
[7] Birnbaum K, Shasha DE, Wang JY et al. A gene expression map of the Arabidopsis root. Science 2003; 302:1956-60.
[8] Honys D, Twell D. Transcriptome analysis of haploid male gametophyte development in Arabidopsis. Genome Biol 2004; 5:R85.
[9] Wuest SE, Vijverberg K, Schmidt A et al. Arabidopsis female gametophyte gene expression map reveals similarities between plant and animal gametes. Curr Biol 2010; 20:506-12.
*[10] Schmid MW, Schmidt A, Klostermeier UC et al. A powerful method for transcriptional profiling of specific cell types in eukaryotes: laser-assisted microdissection and RNA sequencing. PLoS One 2012; 7:e29685.

This study describes the combination LAM and RNA-Seq for transcriptional profiling of rare cell types using the central cell of Arabidopsis as an example. Comparison to ATH1 microarray data reveals a largely improved sensitivity and accuracy. An exploration of a de novo assembly approach suggests that RNA-Seq can also be applied for the study of single cell transcriptomes in non-model organisms.

**[11] Schmidt A, Wuest SE, Vijverberg K et al. Transcriptome analysis of the Arabidopsis megaspore mother cell uncovers the importance of RNA helicases for plant germline development. PLoS Biol 2011; 9:e1001155.

The paper describes the meticulous sampling of megaspore mother cell from the *Arabidopsis* nucellar region by LAM and subsequent transcriptional profiling. Amongst a gene family of RNA helicases with strongly enriched expression in this cell type, the *MNEME* (*MEM*) gene was functionally characterized. *MEM* is shown to be involved in the restriction of the female germline to a single cell in the nucellus, such that *mem* mutations show aspects of apospory, an element of apomixis.

**[12] Tucker MR, Okada T, Hu Y et al. Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in Arabidopsis. Development 2012; 139:1399-404.

The authors used LAM and microarray expression profiling of the nucellar region of developing Arabidopsis ovules. They describe elevated expression of AGO5 in this tissue, and functional analyses of small RNA pathway components suggest that these promote megagametogenesis. The finding complements the previously described role for certain small RNA pathways to restrict the female germline in the nucellus. The resulting model proposes that at least two small RNA pathways are active in ovules, one to restrict and the other to promote the reproductive potential of cells.

[13] Gifford ML, Dean A, Gutierrez RA et al. Cell-specific nitrogen responses mediate developmental plasticity. Proc Natl Acad Sci U S A 2008; 105:803-8.
[14] Dinneny JR, Long TA, Wang JY et al. Cell identity mediates the response of Arabidopsis roots to abiotic stress. Science 2008; 320:942-5.
[15] Long TA, Tsukagoshi H, Busch W et al. The bHLH transcription factor POPEYE regulates response to iron deficiency in Arabidopsis roots. Plant Cell 2010; 22:2219-36.
[16] Pu L, Brady S. Systems biology update: cell type-specific transcriptional regulatory networks. Plant Physiol 2010; 152:411-9.
[17] Galbraith DW, Birnbaum K. Global studies of cell type-specific gene expression in plants. Annu Rev Plant Biol 2006; 57:451-75.
[18] Schmid M, Davison TS, Henz SR et al. A gene expression map of Arabidopsis thaliana development. Nat Genet 2005; 37:501-6.
[19] Long TA. Many needles in a haystack: cell-type specific abiotic stress responses. Curr Opin Plant

Biol 2011; 14:325-31.
[20] Schauer A. Protein dynamics of pollen development. PhD Thesis, University of Zürich; 2010.
[21] Leonhardt N, Kwak JM, Robert N et al. Microarray expression analyses of Arabidopsis guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell 2004; 16:596-615.
[22] Marks MD, Betancur L, Gilding E et al. A new method for isolating large quantities of Arabidopsis trichomes for transcriptome, cell wall and other types of analyses. Plant J 2008; 56:483-92.
[23] Amme S, Rutten T, Melzer M et al. A proteome approach defines protective functions of tobacco leaf trichomes. Proteomics 2005; 5:2508-18.
[24] Zhao Z, Zhang W, Stanley BA, Assmann SM. Functional proteomics of Arabidopsis thaliana guard cells uncovers new stomatal signaling pathways. Plant Cell 2008; 20:3210-26.
[25] Wu T, Wang Y, Guo D. Investigation of glandular trichome proteins in Artemisia annua L. using comparative proteomics. PLoS One 2012; 7:e41822.
[26] Ebert B, Zoller D, Erban A et al. Metabolic profiling of Arabidopsis thaliana epidermal cells. J Exp Bot 2010; 61:1321-35.
[27] Wohrmann HJ, Gagliardini V, Raissig MT et al. Identification of a DNA methylation-independent imprinting control region at the Arabidopsis MEDEA locus. Genes Dev 2012; 26:1837-50.
[28] Bergmann DC, Lukowitz W, Somerville CR. Stomatal development and pattern controlled by a MAPKK kinase. Science 2004; 304:1494-7.
[29] Wellmer F, Riechmann JL, Alves-Ferreira M, Meyerowitz EM. Genome-wide analysis of spatial gene expression in Arabidopsis flowers. Plant Cell 2004; 16:1314-26.
[30] Gomez-Mena C, de Folter S, Costa MM et al. Transcriptional program controlled by the floral homeotic gene AGAMOUS during early organogenesis. Development 2005; 132:429-38.
[31] Wuest SE, O'Maoileidigh DS, Rae L et al. Molecular basis for the specification of floral organs by APETALA3 and PISTILLATA. Proc Natl Acad Sci U S A 2012.
[32] Sanchez-Leon N, Arteaga-Vazquez M, Alvarez-Mejia C et al. Transcriptional analysis of the Arabidopsis ovule by massively parallel signature sequencing. J Exp Bot 2012; 63:3829-42.
[33] Yu HJ, Hogan P, Sundaresan V. Analysis of the female gametophyte transcriptome of Arabidopsis by comparative expression profiling. Plant Physiol 2005; 139:1853-69.
[34] Johnston AJ, Meier P, Gheyselinck J et al. Genetic subtraction profiling identifies genes essential for Arabidopsis reproduction and reveals interaction between the female gametophyte and the maternal sporophyte. Genome Biol 2007; 8:R204.
[35] Jones-Rhoades MW, Borevitz JO, Preuss D. Genome-wide expression profiling of the Arabidopsis female gametophyte identifies families of small, secreted proteins. PLoS Genet 2007; 3:1848-61.
[36] Brady SM, Zhang L, Megraw M et al. A stele-enriched gene regulatory network in the Arabidopsis root. Mol Syst Biol 2011; 7:459.
[37] Zhang C, Barthelson RA, Lambert GM, Galbraith DW. Global characterization of cell-specific gene expression through fluorescence-activated sorting of nuclei. Plant Physiol 2008; 147:30-40.
[38] Nawy T, Lee JY, Colinas J et al. Transcriptional profile of the Arabidopsis root quiescent center. Plant Cell 2005; 17:1908-25.
[39] Petricka JJ, Schauer MA, Megraw M et al. The protein expression landscape of the Arabidopsis root. Proc Natl Acad Sci U S A 2012; 109:6811-8.
[40] Miller MR, Robinson KJ, Cleary MD, Doe CQ. TU-tagging: cell type-specific RNA isolation from intact complex tissues. Nat Methods 2009; 6:439-41.
[41] Doyle JP, Dougherty JD, Heiman M et al. Application of a translational profiling approach for the comparative analysis of CNS cell types. Cell 2008; 135:749-62.
[42] Heiman M, Schaefer A, Gong S et al. A translational profiling approach for the molecular characterization of CNS cell types. Cell 2008; 135:738-48.
[43] Jiao Y, Meyerowitz EM. Cell-type specific analysis of translating RNAs in developing flowers reveals new levels of control. Mol Syst Biol 2010; 6:419.
[44] Mustroph A, Zanetti ME, Jang CJ et al. Profiling translatomes of discrete cell populations resolves altered cellular priorities during hypoxia in Arabidopsis. Proc Natl Acad Sci U S A 2009; 106:18843-8.
**[45] Deal RB, Henikoff S. A simple method for gene expression and chromatin profiling of individual cell types within a tissue. Dev Cell 2010; 18:1030-40.

INTACT, a simple, cheap, and powerful technique to isolate cell-type specific nuclei is described in this paper. It relies on the epitope-tagging of proteins localized to the nuclear periphery and affinity-purification. The method is shown to have high sensitivity and very good yields. It was used for expression as well as chromatin profiling of two root epidermal cell types.

[46] Deal RB, Henikoff S. The INTACT method for cell type-specific gene expression and chromatin profiling in Arabidopsis thaliana. Nat Protoc 2011; 6:56-68.

[47] Steiner FA, Talbert PB, Kasinathan S et al. Cell-type-specific nuclei purification from whole animals for genome-wide expression and chromatin profiling. Genome Res 2012; 22:766-77.
**[48] Henry GL, Davis FP, Picard S, Eddy SR. Cell type-specific genomics of Drosophila neurons. Nucleic Acids Res 2012.

A modified INTACT method is described to isolate tagged nuclei from specific *Drosophila melanogaster* neurons. The authors managed to enrich extremely rare neuronal cell types with unprecedented recoveries and excellent yields for expression as well as chromatin profiling.

[49] Dresselhaus T, Lorz H, Kranz E. Representative cDNA libraries from few plant cells. Plant J 1994; 5:605-10.
[50] Marton ML, Cordts S, Broadhvest J, Dresselhaus T. Micropylar pollen tube guidance by egg apparatus 1 of maize. Science 2005; 307:573-6.
[51] Okuda S, Tsutsui H, Shiina K et al. Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. Nature 2009; 458:357-61.
[52] Brooks L, 3rd, Strable J, Zhang X et al. Microdissection of shoot meristem functional domains. PLoS Genet 2009; 5:e1000476.
[53] Cai S, Lashbrook CC. Stamen abscission zone transcriptome profiling reveals new candidates for abscission control: enhanced retention of floral organs in transgenic plants overexpressing Arabidopsis ZINC FINGER PROTEIN2. Plant Physiol 2008; 146:1305-21.
[54] Deeken R, Ache P, Kajahn I et al. Identification of Arabidopsis thaliana phloem RNAs provides a search criterion for phloem-based transcripts hidden in complex datasets of microarray experiments. Plant J 2008; 55:746-59.
[55] Kerk NM, Ceserani T, Tausta SL et al. Laser capture microdissection of cells from plant tissues. Plant Physiol 2003; 132:27-35.
[56] Le BH, Cheng C, Bui AQ et al. Global analysis of gene activity during Arabidopsis seed development and identification of seed-specific transcription factors. Proc Natl Acad Sci U S A 2010; 107:8063-70.
[57] Ohtsu K, Smith MB, Emrich SJ et al. Global gene expression analysis of the shoot apical meristem of maize (Zea mays L.). Plant J 2007; 52:391-404.
[58] Spencer MW, Casson SA, Lindsey K. Transcriptional profiling of the Arabidopsis embryo. Plant Physiol 2007; 143:924-40.
[59] Walia H, Josefsson C, Dilkes B et al. Dosage-dependent deregulation of an AGAMOUS-LIKE gene cluster contributes to interspecific incompatibility. Curr Biol 2009; 19:1128-32.
[60] You W, Tyczewska A, Spencer M et al. Atypical DNA methylation of genes encoding cysteine-rich peptides in Arabidopsis thaliana. BMC Plant Biol 2012; 12:51.
[61] Dwivedi S, Perotti E, Ortiz R. Towards molecular breeding of reproductive traits in cereal crops. Plant Biotechnol J 2008; 6:529-59.
[62] Koltunow AM, Johnson SD, Okada T. Apomixis in hawkweed: Mendel's experimental nemesis. J Exp Bot 2011; 62:1699-707.
[63] Spillane C, Curtis MD, Grossniklaus U. Apomixis technology development-virgin births in farmers' fields? Nat Biotechnol 2004; 22:687-91.
[64] Ma H, Sundaresan V. Development of flowering plant gametophytes. Curr Top Dev Biol 2010; 91:379-412.
[65] Grossniklaus U, Schneitz K. The molecular and genetic basis of ovule and megagametophyte development. Semin Cell Dev Biol 1998; 9:227-38.
[66] Sprunck S, Gross-Hardt R. Nuclear behavior, cell polarity, and cell specification in the female gametophyte. Sexual Plant Reproduction 2011; 24:123-36.
[67] Chevalier E, Loubert-Hudon A, Zimmerman EL, Matton DP. Cell-cell communication and signalling pathways within the ovule: from its inception to fertilization. New Phytol 2011; 192:13-28.
[68] Brukhin V, Curtis MD, Grossniklaus U. The angiosperm female gametophyte: No longer the forgotten generation. Current Science 2005; 89:1844-1852.
[69] Ning J, Peng XB, Qu LH et al. Differential gene expression in egg cells and zygotes suggests that the transcriptome is restructed before the first zygotic division in tobacco. FEBS Lett 2006; 580:1747-52.
[70] Kumlehn J, Kirik V, Czihal A et al. Parthenogenetic egg cells of wheat: cellular and molecular studies. Sexual Plant Reproduction 2001; 14:239-243.
[71] Krohn NG, Lausser A, Juranic M, Dresselhaus T. Egg Cell Signaling by the Secreted Peptide ZmEAL1 Controls Antipodal Cell Fate. Dev Cell 2012; 23:219-25.
*[72] Koszegi D, Johnston AJ, Rutten T et al. Members of the RKD transcription factor family induce an egg cell-like gene expression program. Plant J 2011; 67:280-91.

The authors describe the egg cell-specific expression of two RWP-RK domain containing transcription factors in wheat (*TaRKD1/2*), discovered by an EST sequencing approach. Similarly, two *Arabidopsis* homologues (*AtRKD1/2*) were found to be expressed specifically in egg cells. Ectopic expression of these two genes in *Arabidopsis* leads to proliferation of undifferentiated cells and the partial reprograming towards an egg cell-specific transcriptional program.

[73] Ferris PJ, Goodenough UW. Mating type in Chlamydomonas is specified by mid, the minus-dominance gene. Genetics 1997; 146:859-69.

*[74] Ohnishi T, Takanashi H, Mogi M et al. Distinct gene expression profiles in egg and synergid cells of rice as revealed by cell type-specific microarrays. Plant Physiol 2011; 155:881-91.

This paper describes a thorough, genome-wide characterization of rice synergid and egg cell transcriptomes, representing the first whole-genome study on the egg apparatus in a monocot species.

[75] Kessler SA, Shimosato-Asano H, Keinath NF et al. Conserved molecular components for pollen tube reception and fungal invasion. Science 2010; 330:968-71.

[76] Leshem Y, Johnson C, Wuest SE et al. Molecular Characterization of the glauce Mutant: A Central Cell-Specific Function Is Required for Double Fertilization in Arabidopsis. Plant Cell 2012.

[77] Sprunck S, Rademacher S, Vogler F et al. Egg cell-secreted EC1 triggers sperm cell activation during double fertilization. Science 2012; in press.

[78] Grossniklaus U. Plant germline development: a tale of cross-talk, signaling, and cellular interactions. Sexual Plant Reproduction 2011; 24:91-5.

**[79] Olmedo-Monfil V, Duran-Figueroa N, Arteaga-Vazquez M et al. Control of female gamete formation by a small RNA pathway in Arabidopsis. Nature 2010; 464:628-32.

This report reveals the role of epigenetic factors *AGO9*, *SGS3* and *RDR6* (all involved in small RNA pathways) during female germline specification. Its results suggest that these genes act to restrict the germline to a single cell, such that mutants in these genes result in a phenotype resembling apospory. Furthermore, in *ago9* mutants transposable elements become expressed during megagametogenesis, suggesting that small pathways are involved in the protection of the plant germline against transposition.

[80] Li S, Pandey S, Gookin TE et al. Gene-sharing networks reveal organizing principles of transcriptomes in Arabidopsis and other multicellular organisms. Plant Cell 2012; 24:1362-78.

[81] Chen C, Farmer AD, Langley RJ et al. Meiosis-specific gene discovery in plants: RNA-Seq applied to isolated Arabidopsis male meiocytes. BMC Plant Biol 2010; 10:280.

[82] Yang H, Lu P, Wang Y, Ma H. The transcriptome landscape of Arabidopsis male meiocytes from high-throughput sequencing: the complexity and evolution of the meiotic process. Plant J 2011; 65:503-16.

[83] Trapnell C, Williams BA, Pertea G et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 2010; 28:511-5.

[84] Filichkin SA, Priest HD, Givan SA et al. Genome-wide mapping of alternative splicing in Arabidopsis thaliana. Genome Res 2010; 20:45-58.

[85] Lister R, O'Malley RC, Tonti-Filippini J et al. Highly integrated single-base resolution maps of the epigenome in Arabidopsis. Cell 2008; 133:523-36.

[86] Song BH, Mitchell-Olds T. Evolutionary and Ecological Genomics of Non-Model Plants. J Syst Evol 2011; 49:17-24.

[87] Szovenyi P, Rensing SA, Lang D et al. Generation-biased gene expression in a bryophyte model system. Mol Biol Evol 2011; 28:803-12.

[88] Der JP, Barker MS, Wickett NJ et al. De novo characterization of the gametophyte transcriptome in bracken fern, Pteridium aquilinum. BMC Genomics 2011; 12:99.

[89] Thiel J, Hollmann J, Rutten T et al. 454 transcriptome sequencing suggests a role for two-component signalling in cellularization and differentiation of barley endosperm transfer cells. PLoS One 2012; 7:e41867.

[90] Bargmann BO, Birnbaum KD. Fluorescence activated cell sorting of plant protoplasts. J Vis Exp 2010.

[1] Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 2009; 10:57-63.
[2] Taylor-Teeples M, Ron M, Brady SM. Novel biological insights revealed from cell type-specific expression profiling. Curr Opin Plant Biol 2011; 14:601-7.
[3] Rogers ED, Jackson T, Moussaieff A, Aharoni A, Benfey PN. Cell type-specific transcriptional profiling: implications for metabolite profiling. Plant J 2012; 70:5-17.
[4] Brady SM, Orlando DA, Lee JY, Wang JY, Koch J, Dinneny JR, Mace D, Ohler U, Benfey PN. A high-resolution root spatiotemporal map reveals dominant expression patterns. Science 2007; 318:801-6.
[5] Moreno-Risueno MA, Van Norman JM, Moreno A, Zhang J, Ahnert SE, Benfey PN. Oscillating gene expression determines competence for periodic Arabidopsis root branching. Science 2010; 329:1306-11.
[6] Iyer-Pascuzzi AS, Jackson T, Cui H, Petricka JJ, Busch W, Tsukagoshi H, Benfey PN. Cell identity regulators link development and stress responses in the Arabidopsis root. Dev Cell 2011; 21:770-82.
[7] Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW, Benfey PN. A gene expression map of the Arabidopsis root. Science 2003; 302:1956-60.
[8] Honys D, Twell D. Transcriptome analysis of haploid male gametophyte development in Arabidopsis. Genome Biol 2004; 5:R85.
[9] Wuest SE, Vijverberg K, Schmidt A, Weiss M, Gheyselinck J, Lohr M, Wellmer F, Rahnenfuhrer J, von Mering C, Grossniklaus U. Arabidopsis female gametophyte gene expression map reveals similarities between plant and animal gametes. Curr Biol 2010; 20:506-12.
[10] Schmid MW, Schmidt A, Klostermeier UC, Barann M, Rosenstiel P, Grossniklaus U. A powerful method for transcriptional profiling of specific cell types in eukaryotes: laser-assisted microdissection and RNA sequencing. PLoS One 2012; 7:e29685.
[11] Schmidt A, Wuest SE, Vijverberg K, Baroux C, Kleen D, Grossniklaus U. Transcriptome analysis of the Arabidopsis megaspore mother cell uncovers the importance of RNA helicases for plant germline development. PLoS Biol 2011; 9:e1001155.
[12] Tucker MR, Okada T, Hu Y, Scholefield A, Taylor JM, Koltunow AM. Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in Arabidopsis. Development 2012; 139:1399-404.
[13] Gifford ML, Dean A, Gutierrez RA, Coruzzi GM, Birnbaum KD. Cell-specific nitrogen responses mediate developmental plasticity. Proc Natl Acad Sci U S A 2008; 105:803-8.
[14] Dinneny JR, Long TA, Wang JY, Jung JW, Mace D, Pointer S, Barron C, Brady SM, Schiefelbein J, Benfey PN. Cell identity mediates the response of Arabidopsis roots to abiotic stress. Science 2008; 320:942-5.
[15] Long TA, Tsukagoshi H, Busch W, Lahner B, Salt DE, Benfey PN. The bHLH transcription factor POPEYE regulates response to iron deficiency in Arabidopsis roots. Plant Cell 2010; 22:2219-36.
[16] Pu L, Brady S. Systems biology update: cell type-specific transcriptional regulatory networks. Plant Physiol 2010; 152:411-9.
[17] Galbraith DW, Birnbaum K. Global studies of cell type-specific gene expression in plants. Annu Rev Plant Biol 2006; 57:451-75.
[18] Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Scholkopf B, Weigel D, Lohmann JU. A gene expression map of Arabidopsis thaliana development. Nat Genet 2005; 37:501-6.
[19] Long TA. Many needles in a haystack: cell-type specific abiotic stress responses. Curr Opin Plant Biol 2011; 14:325-31.
[20] Schauer A. Protein dynamics of pollen development. In: University of Zürich; 2010.
[21] Leonhardt N, Kwak JM, Robert N, Waner D, Leonhardt G, Schroeder JI. Microarray expression analyses of Arabidopsis guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell 2004; 16:596-615.
[22] Marks MD, Betancur L, Gilding E, Chen F, Bauer S, Wenger JP, Dixon RA, Haigler CH. A new method for isolating large quantities of Arabidopsis trichomes for transcriptome, cell wall and other types of analyses. Plant J 2008; 56:483-92.
[23] Amme S, Rutten T, Melzer M, Sonsmann G, Vissers JP, Schlesier B, Mock HP. A proteome approach defines protective functions of tobacco leaf trichomes. Proteomics 2005; 5:2508-18.
[24] Zhao Z, Zhang W, Stanley BA, Assmann SM. Functional proteomics of Arabidopsis thaliana guard cells uncovers new stomatal signaling pathways. Plant Cell 2008; 20:3210-26.
[25] Wu T, Wang Y, Guo D. Investigation of glandular trichome proteins in Artemisia annua L. using comparative proteomics. PLoS One 2012; 7:e41822.

[26] Ebert B, Zoller D, Erban A, Fehrle I, Hartmann J, Niehl A, Kopka J, Fisahn J. Metabolic profiling of Arabidopsis thaliana epidermal cells. J Exp Bot 2010; 61:1321-35.
[27] Wohrmann HJ, Gagliardini V, Raissig MT, Wehrle W, Arand J, Schmidt A, Tierling S, Page DR, Schob H, Walter J, Grossniklaus U. Identification of a DNA methylation-independent imprinting control region at the Arabidopsis MEDEA locus. Genes Dev 2012; 26:1837-50.
[28] Bergmann DC, Lukowitz W, Somerville CR. Stomatal development and pattern controlled by a MAPKK kinase. Science 2004; 304:1494-7.
[29] Wellmer F, Riechmann JL, Alves-Ferreira M, Meyerowitz EM. Genome-wide analysis of spatial gene expression in Arabidopsis flowers. Plant Cell 2004; 16:1314-26.
[30] Gomez-Mena C, de Folter S, Costa MM, Angenent GC, Sablowski R. Transcriptional program controlled by the floral homeotic gene AGAMOUS during early organogenesis. Development 2005; 132:429-38.
[31] Wuest SE, O'Maoileidigh DS, Rae L, Kwasniewska K, Raganelli A, Hanczaryk K, Lohan AJ, Loftus B, Graciet E, Wellmer F. Molecular basis for the specification of floral organs by APETALA3 and PISTILLATA. Proc Natl Acad Sci U S A 2012.
[32] Sanchez-Leon N, Arteaga-Vazquez M, Alvarez-Mejia C, Mendiola-Soto J, Duran-Figueroa N, Rodriguez-Leal D, Rodriguez-Arevalo I, Garcia-Campayo V, Garcia-Aguilar M, Olmedo-Monfil V, Arteaga-Sanchez M *et al*. Transcriptional analysis of the Arabidopsis ovule by massively parallel signature sequencing. J Exp Bot 2012; 63:3829-42.
[33] Yu HJ, Hogan P, Sundaresan V. Analysis of the female gametophyte transcriptome of Arabidopsis by comparative expression profiling. Plant Physiol 2005; 139:1853-69.
[34] Johnston AJ, Meier P, Gheyselinck J, Wuest SE, Federer M, Schlagenhauf E, Becker JD, Grossniklaus U. Genetic subtraction profiling identifies genes essential for Arabidopsis reproduction and reveals interaction between the female gametophyte and the maternal sporophyte. Genome Biol 2007; 8:R204.
[35] Jones-Rhoades MW, Borevitz JO, Preuss D. Genome-wide expression profiling of the Arabidopsis female gametophyte identifies families of small, secreted proteins. PLoS Genet 2007; 3:1848-61.
[36] Brady SM, Zhang L, Megraw M, Martinez NJ, Jiang E, Yi CS, Liu W, Zeng A, Taylor-Teeples M, Kim D, Ahnert S *et al*. A stele-enriched gene regulatory network in the Arabidopsis root. Mol Syst Biol 2011; 7:459.
[37] Zhang C, Barthelson RA, Lambert GM, Galbraith DW. Global characterization of cell-specific gene expression through fluorescence-activated sorting of nuclei. Plant Physiol 2008; 147:30-40.
[38] Nawy T, Lee JY, Colinas J, Wang JY, Thongrod SC, Malamy JE, Birnbaum K, Benfey PN. Transcriptional profile of the Arabidopsis root quiescent center. Plant Cell 2005; 17:1908-25.
[39] Petricka JJ, Schauer MA, Megraw M, Breakfield NW, Thompson JW, Georgiev S, Soderblom EJ, Ohler U, Moseley MA, Grossniklaus U, Benfey PN. The protein expression landscape of the Arabidopsis root. Proc Natl Acad Sci U S A 2012; 109:6811-8.
[40] Miller MR, Robinson KJ, Cleary MD, Doe CQ. TU-tagging: cell type-specific RNA isolation from intact complex tissues. Nat Methods 2009; 6:439-41.
[41] Doyle JP, Dougherty JD, Heiman M, Schmidt EF, Stevens TR, Ma G, Bupp S, Shrestha P, Shah RD, Doughty ML, Gong S *et al*. Application of a translational profiling approach for the comparative analysis of CNS cell types. Cell 2008; 135:749-62.
[42] Heiman M, Schaefer A, Gong S, Peterson JD, Day M, Ramsey KE, Suarez-Farinas M, Schwarz C, Stephan DA, Surmeier DJ, Greengard P *et al*. A translational profiling approach for the molecular characterization of CNS cell types. Cell 2008; 135:738-48.
[43] Jiao Y, Meyerowitz EM. Cell-type specific analysis of translating RNAs in developing flowers reveals new levels of control. Mol Syst Biol 2010; 6:419.
[44] Mustroph A, Zanetti ME, Jang CJ, Holtan HE, Repetti PP, Galbraith DW, Girke T, Bailey-Serres J. Profiling translatomes of discrete cell populations resolves altered cellular priorities during hypoxia in Arabidopsis. Proc Natl Acad Sci U S A 2009; 106:18843-8.
[45] Deal RB, Henikoff S. A simple method for gene expression and chromatin profiling of individual cell types within a tissue. Dev Cell 2010; 18:1030-40.
[46] Deal RB, Henikoff S. The INTACT method for cell type-specific gene expression and chromatin profiling in Arabidopsis thaliana. Nat Protoc 2011; 6:56-68.
[47] Steiner FA, Talbert PB, Kasinathan S, Deal RB, Henikoff S. Cell-type-specific nuclei purification from whole animals for genome-wide expression and chromatin profiling. Genome Res 2012; 22:766-77.
[48] Henry GL, Davis FP, Picard S, Eddy SR. Cell type-specific genomics of Drosophila neurons. Nucleic Acids Res 2012.
[49] Dresselhaus T, Lorz H, Kranz E. Representative cDNA libraries from few plant cells. Plant J 1994;

5:605-10.
[50] Marton ML, Cordts S, Broadhvest J, Dresselhaus T. Micropylar pollen tube guidance by egg apparatus 1 of maize. Science 2005; 307:573-6.
[51] Okuda S, Tsutsui H, Shiina K, Sprunck S, Takeuchi H, Yui R, Kasahara RD, Hamamura Y, Mizukami A, Susaki D, Kawano N *et al*. Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. Nature 2009; 458:357-61.
[52] Brooks L, 3rd, Strable J, Zhang X, Ohtsu K, Zhou R, Sarkar A, Hargreaves S, Elshire RJ, Eudy D, Pawlowska T, Ware D *et al*. Microdissection of shoot meristem functional domains. PLoS Genet 2009; 5:e1000476.
[53] Cai S, Lashbrook CC. Stamen abscission zone transcriptome profiling reveals new candidates for abscission control: enhanced retention of floral organs in transgenic plants overexpressing Arabidopsis ZINC FINGER PROTEIN2. Plant Physiol 2008; 146:1305-21.
[54] Deeken R, Ache P, Kajahn I, Klinkenberg J, Bringmann G, Hedrich R. Identification of Arabidopsis thaliana phloem RNAs provides a search criterion for phloem-based transcripts hidden in complex datasets of microarray experiments. Plant J 2008; 55:746-59.
[55] Kerk NM, Ceserani T, Tausta SL, Sussex IM, Nelson TM. Laser capture microdissection of cells from plant tissues. Plant Physiol 2003; 132:27-35.
[56] Le BH, Cheng C, Bui AQ, Wagmaister JA, Henry KF, Pelletier J, Kwong L, Belmonte M, Kirkbride R, Horvath S, Drews GN *et al*. Global analysis of gene activity during Arabidopsis seed development and identification of seed-specific transcription factors. Proc Natl Acad Sci U S A 2010; 107:8063-70.
[57] Ohtsu K, Smith MB, Emrich SJ, Borsuk LA, Zhou R, Chen T, Zhang X, Timmermans MC, Beck J, Buckner B, Janick-Buckner D *et al*. Global gene expression analysis of the shoot apical meristem of maize (Zea mays L.). Plant J 2007; 52:391-404.
[58] Spencer MW, Casson SA, Lindsey K. Transcriptional profiling of the Arabidopsis embryo. Plant Physiol 2007; 143:924-40.
[59] Walia H, Josefsson C, Dilkes B, Kirkbride R, Harada J, Comai L. Dosage-dependent deregulation of an AGAMOUS-LIKE gene cluster contributes to interspecific incompatibility. Curr Biol 2009; 19:1128-32.
[60] You W, Tyczewska A, Spencer M, Daxinger L, Schmid MW, Grossniklaus U, Simon SA, Meyers BC, Matzke AJ, Matzke M. Atypical DNA methylation of genes encoding cysteine-rich peptides in Arabidopsis thaliana. BMC Plant Biol 2012; 12:51.
[61] Dwivedi S, Perotti E, Ortiz R. Towards molecular breeding of reproductive traits in cereal crops. Plant Biotechnol J 2008; 6:529-59.
[62] Koltunow AM, Johnson SD, Okada T. Apomixis in hawkweed: Mendel's experimental nemesis. J Exp Bot 2011; 62:1699-707.
[63] Spillane C, Curtis MD, Grossniklaus U. Apomixis technology development-virgin births in farmers' fields? Nat Biotechnol 2004; 22:687-91.
[64] Ma H, Sundaresan V. Development of flowering plant gametophytes. Curr Top Dev Biol 2010; 91:379-412.
[65] Grossniklaus U, Schneitz K. The molecular and genetic basis of ovule and megagametophyte development. Semin Cell Dev Biol 1998; 9:227-38.
[66] Sprunck S, Gross-Hardt R. Nuclear behavior, cell polarity, and cell specification in the female gametophyte. Sexual Plant Reproduction 2011; 24:123-36.
[67] Chevalier E, Loubert-Hudon A, Zimmerman EL, Matton DP. Cell-cell communication and signalling pathways within the ovule: from its inception to fertilization. New Phytol 2011; 192:13-28.
[68] Brukhin V, Curtis MD, Grossniklaus U. The angiosperm female gametophyte: No longer the forgotten generation. Current Science 2005; 89:1844-1852.
[69] Ning J, Peng XB, Qu LH, Xin HP, Yan TT, Sun M. Differential gene expression in egg cells and zygotes suggests that the transcriptome is restructed before the first zygotic division in tobacco. FEBS Lett 2006; 580:1747-52.
[70] Kumlehn J, Kirik V, Czihal A, Altschmied L, Matzk F, Lorz H, Baumlein H. Parthenogenetic egg cells of wheat: cellular and molecular studies. Sexual Plant Reproduction 2001; 14:239-243.
[71] Krohn NG, Lausser A, Juranic M, Dresselhaus T. Egg Cell Signaling by the Secreted Peptide ZmEAL1 Controls Antipodal Cell Fate. Dev Cell 2012; 23:219-25.
[72] Koszegi D, Johnston AJ, Rutten T, Czihal A, Altschmied L, Kumlehn J, Wust SE, Kirioukhova O, Gheyselinck J, Grossniklaus U, Baumlein H. Members of the RKD transcription factor family induce an egg cell-like gene expression program. Plant J 2011; 67:280-91.
[73] Ferris PJ, Goodenough UW. Mating type in Chlamydomonas is specified by mid, the minus-dominance gene. Genetics 1997; 146:859-69.

[74] Ohnishi T, Takanashi H, Mogi M, Takahashi H, Kikuchi S, Yano K, Okamoto T, Fujita M, Kurata N, Tsutsumi N. Distinct gene expression profiles in egg and synergid cells of rice as revealed by cell type-specific microarrays. Plant Physiol 2011; 155:881-91.
[75] Kessler SA, Shimosato-Asano H, Keinath NF, Wuest SE, Ingram G, Panstruga R, Grossniklaus U. Conserved molecular components for pollen tube reception and fungal invasion. Science 2010; 330:968-71.
[76] Leshem Y, Johnson C, Wuest SE, Song X, Ngo QA, Grossniklaus U, Sundaresan V. Molecular Characterization of the glauce Mutant: A Central Cell-Specific Function Is Required for Double Fertilization in Arabidopsis. Plant Cell 2012.
[77] Sprunck S, Rademacher S, Vogler F, Gheyselinck J, Grossniklaus U, Dresselhaus T. Egg cell-secreted EC1 triggers sperm cell activation during double fertilization. Science 2012; in press.
[78] Grossniklaus U. Plant germline development: a tale of cross-talk, signaling, and cellular interactions. Sexual Plant Reproduction 2011; 24:91-5.
[79] Olmedo-Monfil V, Duran-Figueroa N, Arteaga-Vazquez M, Demesa-Arevalo E, Autran D, Grimanelli D, Slotkin RK, Martienssen RA, Vielle-Calzada JP. Control of female gamete formation by a small RNA pathway in Arabidopsis. Nature 2010; 464:628-32.
[80] Li S, Pandey S, Gookin TE, Zhao Z, Wilson L, Assmann SM. Gene-sharing networks reveal organizing principles of transcriptomes in Arabidopsis and other multicellular organisms. Plant Cell 2012; 24:1362-78.
[81] Chen C, Farmer AD, Langley RJ, Mudge J, Crow JA, May GD, Huntley J, Smith AG, Retzel EF. Meiosis-specific gene discovery in plants: RNA-Seq applied to isolated Arabidopsis male meiocytes. BMC Plant Biol 2010; 10:280.
[82] Yang H, Lu P, Wang Y, Ma H. The transcriptome landscape of Arabidopsis male meiocytes from high-throughput sequencing: the complexity and evolution of the meiotic process. Plant J 2011; 65:503-16.
[83] Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 2010; 28:511-5.
[84] Filichkin SA, Priest HD, Givan SA, Shen R, Bryant DW, Fox SE, Wong WK, Mockler TC. Genome-wide mapping of alternative splicing in Arabidopsis thaliana. Genome Res 2010; 20:45-58.
[85] Lister R, O'Malley RC, Tonti-Filippini J, Gregory BD, Berry CC, Millar AH, Ecker JR. Highly integrated single-base resolution maps of the epigenome in Arabidopsis. Cell 2008; 133:523-36.
[86] Song BH, Mitchell-Olds T. Evolutionary and Ecological Genomics of Non-Model Plants. J Syst Evol 2011; 49:17-24.
[87] Szovenyi P, Rensing SA, Lang D, Wray GA, Shaw AJ. Generation-biased gene expression in a bryophyte model system. Mol Biol Evol 2011; 28:803-12.
[88] Der JP, Barker MS, Wickett NJ, dePamphilis CW, Wolf PG. De novo characterization of the gametophyte transcriptome in bracken fern, Pteridium aquilinum. BMC Genomics 2011; 12:99.
[89] Thiel J, Hollmann J, Rutten T, Weber H, Scholz U, Weschke W. 454 transcriptome sequencing suggests a role for two-component signalling in cellularization and differentiation of barley endosperm transfer cells. PLoS One 2012; 7:e41867.
[90] Bargmann BO, Birnbaum KD. Fluorescence activated cell sorting of plant protoplasts. J Vis Exp 2010.